STANDARDIZATION OF HERBAL MEDICINES – AN OVERVIEW

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In the few decades, there has been exponentional growth in the field of herbal medicines. Most of the traditional systems of medicine are effective due to lack of standardization. So there is a need to develop a standardization technique. Standardization of herbal formulation is essential in order to assess the quality, purity, safety and efficacy of the drug. There is increasing awareness and general acceptability of the use herbal drugs in today's medical practice. The world population depends on herbal medicines and product for healthy living. This rise in the use of herbal product has also given rise to various forms of adulteration of the products, which leading to consumers' and manufacturers' disappointment and in some instances fatal consequences. The challenge is innumerable and enormous, to fulfill the need of global herbal market. The standardization of this formulations like the organoleptic characters, physical properties, the various physic-chemical properties such as moisture content, ash values, extractive values need to be carried out along with Thin layer chromatography and heavy metal content study should also carried out to ascertain the quality, purity and safety of this herbal formulation.

Key words: Standardization, quality, purity, herbal products.

INTRODUCTION

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history[1]. It is very important that, a system of standardization is established for every plant medicines in the market; because, the scope for variation in different batches of medicine is enormous. The phytochemical content of plant materials may vary based on places and time of collection, which finally influence therapeutic efficiency. Different environmental factors and cultivation techniques also play important role for production of particular medicinal plants. This indicate the need of suitable quality control test for the entire preparation to ensure quality of the product[2,3,4]. As commercialization of the herbal medicines is going on, assurance of safety, quality and efficacy become an important issue. The herbal raw materials are prone to a lot of variation due to several factors, the important one is, being the identity of the plants and seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotics. Standardization, as defined by American Herbal Product association: "Standardization refers to the body of information and control necessary to product material of reasonable consistency. This achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes.

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs. The fingerprint profiles serve as guideline to the phytochemical profiling of the drug in ensuring the quality, while quantification of the marker compound/s would serve as an additional parameter in assessing the quality of the sample[5,6]. Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced due to contaminants and adulteration. However, some of the plants used in herbal medicines can also be highly toxic. As a whole, herbal medicines can have a risk of adverse effects and drug–drug and/or drug–food

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interactions if not properly assessed. Assessment of the safety of herbal products, therefore, is the first priority in herbal research.

There are various approaches to the evaluation of safety of herbal medicines. The toxic effects of herbal preparation may be attributed mainly to the following:

• Inherent toxicity of plant constituents and ingredients
• Manufacturing malpractice and contamination

Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phytochemical and pharmacological studies. It is, however, safe to assume that, based on human experiences in various cultures, the use of toxic plant ingredients has already been largely eliminated and recent reports of toxicity could largely be due to misidentification and overdosing of certain constituents[7].

World Health Organization (WHO) has defined herbal medicines as finished, labeled medicinal products that contain active ingredients, aerial or underground parts of the plant or other plant materials or combinations. WHO has set specific guidelines for the assessment of the safety, efficacy, and quality of herbal medicines. WHO estimates that approx 81% of the world populations presently use herbal medicines for primary health care[8]. WHO stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles. If a principle active component is known, it is most logical to quantitate these compounds. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose[5,9].

HERBAL MEDICINE

An herb is a plant or part of a plant valued for its medicinal, aromatic or savory qualities. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal remedies or medicines consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied topically to the skin. Herbal products often contain a variety of naturally-occurring biochemical's from plants, many of which contribute to the plant's medicinal benefits. Chemicals known to have medicinal benefits are referred to as "active ingredients" or "active principles" and their presence depends on a number of factors including the plant species, the time and season of harvest, the type of soil, the way the herb is prepared, etc.[1]

Herbs include crude plant materials, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir-baking with honey, alcoholic beverages or other materials.

Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term "mixture herbal product" can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal products to which chemically defined active substances have been added, including synthetic compounds and/or isolated

constituents from herbal materials, are not considered to be herbal[5,10] .

ADVANTAGES OF HERBAL MEDICINE

1. They have large amount of use.
2. They have better patient tolerance as well as acceptance.
3. The medicinal plants have renewable source of cheaper medicines.
4. Improvements in the quality, efficacy and safety of herbal medicines with the development of science and technology.
5. Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
6. They are cheap in cost.
7. They are not harmful.
8. They are more effective than any synthetic drug.
9. Throughout the world herbal medicines have provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form as well as a pure chemical upon which modern medicines are constructed.[11,12]

SOURCES OF TOXIC CHEMICALS AND CONTAMINATION IN HERBAL PRODUCT

The practices of most ethnic herbal medicines include the use of crude or raw herbs that are collected from the wild or from cultivated fields and their prepared or ready-made (formulated mixture of herbal or other natural materials) products. Toxic contaminants may come from:

- Environments and conditions that the medicinal plants are grown or collected.
- The conditions under which they are dried and processed.
- The storage conditions and conditions during transport.
- The manufacturing processes when the ready-made medicinal products are produced.

WHO has also paid serious attention on mycotoxin contamination in herbal drugs, considering it as a global problem. Detection of mycotoxins is certainly a matter of great concern in stored drugs of important medicinal plants.

Table I List of drug containing mycotoxins

DRUG	Concentration of mycotoxins (mg/g)
Roots/ rhizomes of *Asparagus racemosus*	0.16
Atropa belladonna	0.27
Withania somnifera	0.68
Plumbago zelanica	1.13
Terminalia chebula	1.19
Seeds of *Macuna puriens*	1.16

Such herbal drugs containing mycotoxins above the tolerance limit fixed by WHO for human consumption, will be certainly rejected in the global market.[13-15]

PROBLEMS SPECIFIC TO THE QUALITY OF HERBAL DRUGS

- Herbal drugs are mixtures of many constituents
- The active principle(s) is (are) hardly known
- Selective analytical methods may not yet exist
- Reference compounds may not be available commercially
- Chemical variability of plant material
- Natural variation/biodiversity
- Chemovarieties (eg, Thyme) and Chemocultivars (eg, Chamomile)
- Influence of harvest, drying, and storage conditions
- Influence of processing (different extracts: polarity of solvent, mode of extraction, instability of constituents)[16]

NEED OF STANDARDIZATION

The quality control of herbal crude drug & formulation is important in justifying their acceptability in modern system of medicines. Standardization of synthetic drugs offers no problem with very well defined parameters of analysis. It is not uncommon to have as many as five or more different herbal ingredients in one single formulation. The batch to batch variation starts from the collection of the raw materials itself in absence of any reference standard for identification. WHO has emphasized the need to ensure quality control of medicinal plants products by using modern

techniques and by applying suitable standards and parameters. Standardized products and services are valuable User confidence builders' being perceived as

- Safe & Healthy
- Secure
- High Quality
- Flexible

Standardization brings important benefits to business including a solid foundation upon which new technologies are developed and an opportunity to share and enhance existing practices. Standardization also plays a pivotal role in assisting Governments, Administrations, Regulators and the legal profession as legislation, regulation and policy initiatives are all supported by standardization.[17]

PROTOCOLS FOR STANDARDIZATION OF HERBAL EXTRACTS

Table II Parameters for standardization of herbs

Parameter	Specification
Authentication	Identification of plant species with Latin binomial name Steps involves taxonomic, macroscopic and microscopic studies. Stage of collection, part of the plant, regional status, botanical identity such as phytomorphology, microscopical and histological analysis, taxonomical identity etc.
Physical parameters	Physical tests include organoleptic evaluation, viscosity, moisture content, pH, disintegration time, hardness, ash value etc.
Chromatographic and spectroscopic evaluation	Sophisticated modern techniques of standardization such as UV, FTIR, HPTLC, HPLC, GCMS,LCMS, NMR
Microbiological parameters	Microbial contamination can be measured according to validated or pharmacopeia methodology. Example: *E. coli* and molds, total enteriobacterial, aflatoxin
Pesticide residue analysis	Standard limits of pesticides as per WHO and FAO (Food and Agricultural Organization. Example: DDT, BHC, toxaphene and aldrin
Heavy metal analysis	Toxic metals such as Mercury(Hg), Lead (Pb), Cadmium (Cd), Arsenic (As), Copper (Cu), Iron (Fe), Zinc (Zn) as per specifications[7,18].

GUIDELINES FOR HERBAL DRUG STANDARDIZATION (WHO guidelines)

The subject of herbal drug standardization is massively wide and deep. The guidelines set by WHO can be summarized as follows

- Reference to the identity of the drug. Botanical evaluation- sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements etc.
- Reference to the physicochemical character of the drug. Physical and chemical identity, chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil and alkaloidal assays, quantitative estimation protocols etc.
- Reference to the pharmacological parameters, biological activity profiles, bitterness values, haemolytic index, astringency, swelling factor, foaming index etc.
- Toxicity details- pesticide residues, heavy metals, microbial contamination like total viable count, pathogens like *E.coli*, Salmonalla, *P.aeroginosa*, *S. aureus*, Enterobacteria etc.
- Microbial contamination.
- Radioactive contamination.[19]

DOCUMENTED OR REGULATORY APPROACHES FOR HERBAL MEDICINES

Herbal medicines were first included in the WHO International Conference on Drug Regulatory Authorities in 1986. In 1991, WHO prepared draft guidelines for assessment of herbal medicines, which

were adopted by the 6th International Conference on Drug Regulatory Authorities (ICDRA). This includes basic criteria for quality, safety and efficacy of herbal medicines. These guidelines provide valuable assistance to national regulatory authorities, scientific organizations and manufacturers to undertake assessment of documentation of submissions. Following the recommendations of the 6th ICDRA in 1991, WHO continued to develop pharmaceutical monographs on herbal medicines on the basis of guidelines for the assessment of herbal medicines: Part I: Botanical Characteristics, Major Active Constituents, and Quality Control (QC); and Part II: Summaries of Clinical Applications, Pharmacology, Precautions, and Adverse Reactions.

The purpose of the WHO monographs was to

- Provide scientific information on safety, efficacy, and quality control of medicinal plants,
- Facilitate proper use of herbal medicines.

Documentation of herbal medicine should involve documentation on the cultivation, harvesting, and technologies involved, including: plantation development and processing methods; the prior validation of products used in herbal medicine; documentation of the properties of synthetic products identical with or related to the active constituent(s) of the medicine; the chemistry of herbs believed to be responsible for the activity; the results of any clinical trials carried out on the product and aspects of marketing and trading; and legal issues including IPR. This is an unenviable task as only a fraction of the hundreds of thousands of plant species has been fully investigated in the laboratory.

In United States, herbal products can only be marketed as food supplements. Specific health claims need U.S. Food and Drug Administration (FDA) approval. The European Guidelines for the Assessment of Herbal Medicines state that a substance's historical use is valid to document safety and efficacy, in the absence of scientific evidence to the contrary.

Two features of the European approach are as follows:

- Costs are less, it takes less time to approve herbal medicines as safe and effective, and one can apply the "doctrine of reasonable certainty", which does not compromise safety.
- No inherent prejudice exists against complex plant substances; they are considered safe and effective[7,20].

TRADITIONAL APPROACHES FOR STANDARDIZATION OF HERBAL MEDICINES

Methods for quality control of herbal medicines involve sensory inspection (macroscopic and microscopic examinations). Macroscopic identity of botanical materials is based on parameters like shape, size, colour, texture, surface characteristics, fracture characteristics, odour, taste and such organoleptic properties that are compared to a standard reference material. Microscopy involves comparative microscopic inspection of broken as well as powdered, crude, botanical materials and analytical inspection using instrumental techniques such as thin layer chromatography, HPLC, GC.MS, LC.MS, near infrared (NIR), and spectrophotometry etc. Analytical HPLC whereas both the degree of solute purity as well as the amount of compound that can be produced per unit time i.e. throughput or recovery in preparative HPLC [15, 22-23].

HERBAL MEDICINE REGULATION IN EU, US AND INDIA

Table III Regulation for Herbal Medicine

Country	Regulatory authority	Description	Regulation/Act
EU	European Medicines Agency (EMEA): The Committee on Herbal Medicinal Products (HMPC)	Establishment of HMPC and regulation of herbal medicine Registration Procedure for traditional herbal medicinal	Directive 2004/24/EC (Traditional Herbal Medicinal Products Directive) and Regulation (EC) No 726/2004. Articles 16a to 16i of Directive

		products	2001/83/EC
US	USFDA: Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER)	Botanical drug definition Regulation of herbal product Procedure for marketing of Botanical drug as OTC drug Regulation of Allergenic extracts and vaccines that contain botanical ingredients	201(g)(1)(B),Federal Food, Drug, and Cosmetic Act, Dietary Supplement Health and Education Act of 1994 (DSHEA) 21 CFR 10.20, 10.30, 312, 314, 321, 324, 330, 331–358 Section 351 of the Public Health Service Act (42 U.S.C. 262)
India	Department of. Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH)	Production and marketing of ASU drugs GMP for ASU drugs	Drugs & Cosmetics Act, 1940 Drugs & Cosmetics Rules, 1945 Schedule T, Drugs & Cosmetics Act, 1940 .21

CHROMATOGRAPHIC METHODS

Chemical and chromatographic techniques may be used to aid in identification of an herbal material or extract. Chromatographic technique such as HPLC, TLC, GC and capillary electrophoresis and spectroscopic methods such as IR, NMR, and UV may also be used for fingerprinting. DNA fingerprinting has been widely used in many species, e.g. DNA fingerprinting of Panax species and their adulterants. Marker compounds may be used to help identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes and obtain stability profiles. Central Council for Research in Ayurveda and Siddha gave list of phytoconstituents which is used for analysis of crude drugs or herbal formulations containing these crude drugs[22, 24].

TLC

TLC is a simple, low-cost, versatile and specific method for the identification of herbal medicines. The unique feature of picture-like image of TLC supplies an intuitive visible profiling.[24] Nowadays HPTLC is a routine analytical technique. It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC

It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation. With this technique, authentication of various species of plant possible, as well as the evaluation of stability and consistency of their preparations from different manufactures[22, 25].

HPLC

Preparative and analytical HPLC are widely used in pharmaceutical industry for isolating and purification of herbal compounds. There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar). The important parameters to be considered are resolution, sensitivity and fast analysis time[19]. HPLC has been employed to analyze several components in a medicinal preparation composed of several crude drugs [35,36,37]. Among the analytical methods for standardization of Indian herbal medicines HPLC is the most popular one, due to its versatility, precision and relatively low cost, which represents a progress in comparison of the one or two marker quantitative approach. One of the main advantages of HPLC is that many detectors can be coupled with it, such as UV, DAD, ELSD, FLD, RID, MS, and NMR, etc., which supplies much more possibilities for detecting different

constituent types. In recent years, coulometric electrode array detection (HPLCCEAD) and charged aerosol detection (CAD) have been also introduced to the analysis of herbal formulations[22, 26.]

GC AND HYPHENATED TECHNIQUES

GC and GC-MS are unanimously accepted methods for the analysis of volatile constituents of herbal medicines, due to their sensitivity, stability and high efficiency. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the complex constituents. In recent years so many workers developed GC or GC hyphenated with MS for the analysis of phytoconstituents such as thymol, eucalyptol, menthol, camphor from honey and bees wax (Gas chromatography with flame ionisation detection), estragole, safrole and eugenol methyl ether in food products (Gas- chromatograph directly interfaced with GCQ plus mass spectrometer) , eugenol (GC with flame ionization detector) and sugars (Gas chromatograph interfaced with mass selective detector).[27]

CONCLUSION

The field of the herbal drugs and formulations is very vast and there is still lot to explore on the subject of standardization of these. So, while developing an herbal drug formulation it is must to have all the related knowledge of that particular drug including all its organoleptic characters to

phytoconstituents to pharmacological action to its standardization in respect to various parameters via various techniques. Monographs as compiled in the standard books like Indian Pharmacopoeia, Ayurvedic Pharmacopoeia of India, Wealth of India and Ayurvedic formulary, provide all the details for the various tests to be performed in order to determine the conformity of the crude or formulated herbal drug with the standards lay. It is also important to study the influence of the variousfactors like effect of the environment, climate, growth conditions and condition of the storage on the potency of a crude drug or the formulation prepared using it as a whole or as extract or the constituent isolated. It is also important to standardize, not only the main drug constituent but also the other excipients and additives incorporated.

Every day a new chemical entity is being identified and isolated from the existed or newly identified crude drugs, so a need of the stringent regulation has arise to determine the conformity of these new chemicals to assess their physicochemical, pharmacological, clinical activities along with their safety and efficacy.

REFERENCE

1. Kunle, Folashade O, Egharevba, Omoregie H and Ahmadu, Ochogu P, Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation, 2012; 4(3): 101-112.
2. Ekka N R, Namdeo K P and Samal P K, Standardization Strategies for Herbal Drugs-An Overview, Research J. Pharm. and Tech. 2008; 1(4): 310-312.
3. Chaudhury RR, Herbal medicine for human health. World Health Organization Geneva, CBS publishers and distributors LTD New Delhi, 1999.
4. Raina MK, Quality control of herbal and herbo-mineral formulations. Indian journal of natural products, 2003; 19: 11-15.
5. Pravin H, Nikam, Kareparamban J, Jadhav A and Kadam V, Future Trends in Standardization of Herbal Drugs, Journal of Applied Pharmaceutical Science. 2012; 2 (6): 38-44.
6. Sharma V, Agarwal K, Maithani J and Nanda D. Development of Quality Standards of Vatari Churna: A Polyherbal Formulation, IJPSR, 2012; 3(8): 2870-2872.
7. Mosihuzzaman M., and Choudhary M I, Protocols on Safety, Efficacy, Standardization, and Documentation of Herbal Medicine, Pure Appl. Chem. 2008; 80(10): 2195–2230.
8. Nainwal P., Pharmacovigilance of Herbal Medicines: An Intangible Approach, IJPSR, 2010; 1(11): 60-65.
9. Dixit V.K., Yadav N.P. Recent approaches in herbal drug standardization. Integr Biol. 2008; 2 (3): 195-203.

10. General guidelines for methodologies on research and evaluation of traditional medicine. Geneva, World Health Organization, 2000 (WHO/EDM/TRM/2000.1)
11. Zhang X, Twarog S, Kapoor P, traditional medicine: its importance and production and Protecting and promoting traditional knowledge: system, National Experience and International Dimensions, Part 1. The role of traditional knowledge in Helth Care and Agriculture, United nation. New York document. 2002-04; 3-6. UNCTAD/DITC/TED/10.
12. Gogtay N J, Bhatt H A, Dalvi S S, and Kshirsagar N A, The Use and Safety of Non-Alopathic Indian Medicines, Drug Safety,2002;. 25(14): 1005- 1019.
13. .Roy A K, Threat to medicinal plants and drugs by fungi. J. Indian Bot. Soc 1989; 68: 149-153.
14. Dubey N K, Rajesh Kumar and Pramila Tripathi. Global promotion of herbal medicine: India.s opportunity. Current Science 2004; 86 (1): 37-41.
15. Raghavendra H L,Yogesh H S, Gopalakrishna B, Chandrashekhar V M, Sathish B P, An Overview of Herbal Medicine, Int. J. Ph. Sci, 2009;1(1):1-20
16. Rudolf B, Quality Criteria and Standardization o f Phytopharmaceuticals: Can Acceptable Drug Standards Be Achieved? Drug Information Journal, 1998; 32: 101–110
17. Dasgupta P, and De A, Comparative Standardization Study of Two Marketed Ashwagandha Churna Formulation, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012; 3 (2): 734-741.
18. Gautam A, Kashyap S J, Sharma P K, Garg V K, Visht S, Kumar N, Identification, evaluation and standardization of herbal drugs: A review, Der Pharmacia Lettre, 2010; 2(6): 302-315
19. Gatkal S, Punde A, Balap A, and Chaudhari,P, Safety of Herbal Medicine: A Review, International Journal of Pharmaceutical and Chemical Sciences, 2012; 1(4): 1625-1639.
20. ICDRA. International Conference on Drug Regulatory Authorities, World Health Organization (1986).
21. Sahoo N, Manchikanti P, Dey S, Herbal drugs: Standards and regulation, Fitoterapia. 2010; 81: 462–471.
22. Patra K C, Pareta S K, Harwansh R K, Kumar K. J, Traditional approaches towards standardization of herbal medicines -a review, Journal of Pharmaceutical Science and Technology. 2010; 2 (11):372-379
23. Thirumalai D, Paridhavi M, Gowtham M, An Overview of standardization of herbal drugs, Int.J.Rev.Life Sci, 2011;1(3):167-170.
24. Shaw P. C., P. P. Butt. Authentication of Panax species and their adulterants by random primed polymerase chain reaction, Planta Med, 1995; 61: 466-469.
25. Jianga Y., B. David, P. Tu, Y. Barbin. Recent analytical approaches in quality control of traditional Chinese medicines—A review, Anal. Chim. Acta. 2010; 657: 9–18.
26. Bai C.C., S.Y. Han, X.Y. Chai, Y. Jiang, P. Li, P.F. Tu, Sensitive determination of saponins in Radix et Rhizoma Notoginseng by charged aerosol detector coupled with HPLC, J. Liq. Chromatogr. Relat.Technol. 2009; 32: 242-260.
27. Myinff S., W. R.W. Daud, A.B. Mohamad, A. A.H. Kadhum. Gas chromatographic determination of eugenol in ethanol extract of cloves, J. Chromatogr. B, 1996; 679: 193-195.

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